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[Open Access](#)**Lab-on-a-chip-based PCR-RFLP assay for the detection of Malayan box turtle (Cuora amboinensis) in the food chain and traditional Chinese medicines** (Article)Asing^a, [Eaqub Ali, Md.^{ab}](#), [Hamid, S.B.A.^a](#), [Motalib Hossain, M.A.^a](#), [Mustafa, S.](#), [Abdul Kader, Md.^d](#), [Zaidul, I.S.M.^e](#)^a Nanotechnology and Catalysis Research Center (NANOCAT), University of Malaya, Kuala Lumpur, Malaysia^b Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Kuala Lumpur, Malaysia^c Institute of Halal Products Research, University of Putra Malaysia, UPM Serdang, Selangor, Malaysia[View additional affiliations](#)[View references \(101\)](#)

Abstract

The Malayan box turtle (*Cuora amboinensis*) (MBT) is a vulnerable and protected turtle species, but it is a lucrative item in the illegal wildlife trade because of its great appeal as an exotic food item and in traditional medicine. Although several polymerase chain reaction (PCR) assays to identify MBT by various routes have been documented, their applicability for forensic authentication remains inconclusive due to the long length of the amplicon targets, which are easily broken down by natural decomposition, environmental stresses or physiochemical treatments during food processing. To address this research gap, we developed, for the first time, a species-specific PCR-restriction fragment length polymorphism (RFLP) assay with a very short target length (120 bp) to detect MBT in the food chain; this authentication ensured better security and reliability through molecular fingerprints. The PCR-amplified product was digested with Bfa1 endonuclease, and distinctive restriction fingerprints (72, 43 and 5 bp) for MBT were found upon separation in a microfluidic chip-based automated electrophoresis system, which enhances the resolution of short oligos. The chances of any false negative identifications were eliminated through the use of a universal endogenous control for eukaryotes, and the limit of detection was 0.0001 ng DNA or 0.01% of the meat under admixed states. Finally, the optimized PCR-RFLP assay was validated for the screening of raw and processed commercial meatballs, burgers and frankfurters, which are very popular in most countries. The optimized PCR-RFLP assay was further used to screen MBT materials in 153 traditional Chinese medicines of 17 different brands and 62 of them were found MBT positive; wherein the ingredients were not declared in product labels. Overall, the novel assay demonstrated sufficient merit for use in any forensic and/or archaeological authentication of MBT, even under a state of decomposition. © 2016 Asing et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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